MANAGEMENT OF AVIAN BOTULISM AND SURVIVAL OF MOLTING MALLARDS

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ABSTRACT

Avian botulism outbreaks are perpetuated by proliferation of toxin producing *Clostridium botulinum* in bird carcasses and consumption of maggots containing toxin by healthy birds. Removal and disposal of bird carcasses has been advocated for management of outbreaks but this technique is expensive and its effect on reducing waterfowl mortality under field conditions is unknown. Therefore, I radio-marked 335 molting (new primaries <10 mm) mallards (*Anas platyrhynchos*) on 11 lakes in western Canada during midsummer 1999-2001, and monitored their survival for 30 days to evaluate whether survival was greater on lakes with carcass removal. Botulism occurred on 10 of the lakes. On five removal (treatment) lakes greater-than-normal effort was made to remove carcasses as soon as dead birds were detected. On six non-removal (control) lakes no carcasses were removed. In 1999, estimated 30-day survival probability was 4.6% (SE = 0.035) on one large wetland with removal and 4.3% (SE = 0.067) and 38.6% (SE = 0.131) on two wetlands lacking removal. In 2000, estimated survival probability was 35.1% (SE = 0.129) and 70.5% (SE = 0.082) on two removal lakes, and 54.3% (SE = 0.092) and 70.0% (SE = 0.092) on two non-removal lakes. In 2001, botulism was detected on two non-removal lakes where survival probabilities were 85.3% (SE = 0.068) and 86.0% (SE = 0.065), and on one removal lake where the survival probability was 96.2% (SE = 0.037), but not detected on the other removal lake on which no marked birds died from botulism. Overall, when data were organized by carcass removal versus non removal, mallard survival was no greater on lakes where carcasses were removed.

It has been hypothesized that survival of mallards can be improved by reducing carcass densities to <<12/ha. Thus, carcass searches were conducted at dead and random live radio-marked bird locations (n=197). Mean carcass density was higher at dead bird locations ($\bar{x}$ = 11.6, SE = 0.986) than at live bird locations ($\bar{x}$ = 5.0, SE = 0.658). Predicted survival probability rapidly decreased from 0 to 9 carcasses/hectare on 'high risk' lakes, these being lakes with very poor survival, and gradually decreased from 0 to 20 carcasses/hectare on 'low risk' lakes. Density of

maggot-laden carcasses was a better predictor of survival probability than either total or maggot free carcass densities. Differences between low and high risk lakes in the relationship between survival probability and carcass density is likely related to abiotic and biotic factors giving rise to botulism outbreaks.

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Dedicated to Dennis Evelsizer

for introducing me to the outdoors

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CHAPTER 1. GENERAL INTRODUCTION

1.1 AVIAN BOTULISM

CLOSTRIDIUM BOTULINUM (TYPE C, hereafter, botulism or avian botulism), is a naturally occurring bacterium with worldwide distribution and causes food poisoning that results in substantial mortality in birds, notably waterfowl (Hunter et al. 1970, Martinovich et al. 1972, Smith 1976, Samuel 1992). Botulism has caused enormous losses of North American waterfowl, with estimates in the tens of thousands to millions of birds annually (Rocke et al. 1999). During 1994 - 2001, major outbreaks in western Canada occurred primarily on three large prairie lakes, but many wetlands in prairie Canada also had smaller outbreaks (Canadian Cooperative Wildlife Health Centre, pers comm.).

Spores are the dormant stage of the botulism bacteria and have been recovered in the mud of many wetlands (Mitchell and Rosendal 1987, Wobeser et al. 1987). Spores are resistant to heating and drying and can persist in marsh sediments for years (Smith et al. 1982, Wobeser et al. 1987), resembling a "sit and wait" (Ewald 1995) parasite. Waterfowl and other vertebrates that live in wetlands frequently ingest these spores (Haagsma 1974, Smith and Turner 1987, Hubalek and Halouzka 1991, Reed and Rocke 1992). When such an animal dies for any reason and begins to decompose, it may be carrying spores in the liver or gut (Smith and Turner 1987, Reed and Rocke 1992). When an animal dies with spores in its tissues and decomposes, *C. botulinum* may germinate undergo vegetative growth and invade other tissues throughout the carcass. A neurotoxin is produced only in the presence of a specific bacteriophage (virus) (Eklund et al. 1987) which infects the bacteria with the type C_1 toxin gene needed for toxin production. Little is known about the bacteriophage; Williamson et al. (1999) detected it in 16 of 18 wetlands sampled, suggesting it also is usually present in wetlands. Bacteriophages replicate with the vegetative cell growth of the bacteria and without this infection the bacteria cannot produce neurotoxin (Rocke and Samuel 1999). *C.*

botulinum is harmless in the spore state, because neurotoxin is produced only after the spores germinate into vegetative cells and the cells begin multiplying.

Decaying carcasses are considered to provide a suitable substrate and represent the most common source for bacterial growth and toxin production (Hunter et al. 1970, Wobeser and Galmut 1984, Smith and Turner 1987). Although some studies suggest toxin production may occur in invertebrate carcasses, there is no evidence that decaying invertebrates initiate botulism outbreaks (Jensen and Allen 1960, Rocke et al. 1999). Vertebrate carcasses are particularly suitable for bacterial growth and toxin production because they provide a large amount of substrate, a self-contained anaerobic environment (Smith and Turner 1987), and the high temperature needed for optimal growth and toxin production (Wobeser and Galmut 1984).

Botulinum toxin is produced only when environmental conditions are suitable for spore germination and vegetative growth (Rocke et al. 1999). However, the most suitable conditions for bacterial growth and toxin production in wetlands and the ecological features that precipitate outbreaks remain unclear. Outbreaks of botulism in waterfowl are sporadic and unpredictable, occurring annually in some wetlands, but not in adjacent wetlands. Furthermore, the distribution and frequency of outbreaks have increased (Rocke and Samuel 1999), and outbreaks have been documented in late winter or spring (Wobeser 1997). Barras and Kadlec (2000) report that large outbreaks in the Bear River Delta, Utah, were associated with high precipitation and increased water flow. Rocke et al. (1999) reported that outbreak wetlands had significantly lower oxidation-reduction (redox) potential than nonoutbreak wetlands. Rocke and Samuel (1999) found that outbreak wetlands had greater percent organic matter in the sediment and lower redox potential in the water than paired nonoutbreak wetlands. These two studies indicate that risk of botulism increased when redox potential decreased but the mechanism by which redox potential influences outbreaks is unknown. Both studies concluded that other factors such as water temperature, pH, water turbidity, and salinity may be associated with outbreaks but the relationship is complex and may have a more proximate effect in initiating botulism outbreaks.

Toxin produced in carcasses is transferred to other birds when they consume fly maggots and other invertebrate carrion feeders (Wobeser 1997, Rocke et al. 1999). Live invertebrates feeding on decaying carcasses are not susceptible to the toxin, but serve as carriers of toxin (Shillinger and Morley 1937, Lee et al. 1962). Maggots from carcasses are considered to be the major source of toxin during outbreaks (Hunter et al. 1970). Toxic maggots from carcasses of animals dead of any cause might initiate a botulism outbreak; in turn, birds that eat toxic maggots and die of botulism provide substrate for further toxic maggot production causing a carcass-maggot cycle or "propagation stage" in a botulism epidemic (Wobeser and Bollinger 2002, unpubl.). At the propagation stage, botulism can act like an infectious disease (i.e., have properties such as threshold densities, contact rates, etc.) because toxin produced within its victims leads to secondary poisoning of other birds (Wobeser 1997).

1.2 BOTULISM MANAGEMENT

Botulism has been recognized as a food poisoning killing waterfowl since 1930 (Wobeser 1997). Historical methods of managing botulism were designed to reduce the production of toxin and the exposure of birds to toxin. Early attempts to achieve this goal included habitat manipulation through pond construction and water level management (Hunter et al. 1970, and Rosen 1971b), but these methods proved impractical over large areas. More recently, the commonly advocated method of "managing" botulism outbreaks is to conduct surveillance of wetlands during spring and summer, and to collect and dispose of carcasses during botulism outbreaks (Locke and Friend 1987a, Reed and Rocke 1992, Rocke and Samuel 1999). It is believed that bird losses may be reduced by removing carcasses before maggots develop, thus breaking the cycle by reducing the probability of uninfected birds ingesting toxic maggots. This practice can be expensive and time demanding (Wobeser 1987, Friend 1992). During years of severe outbreaks in prairie Canada, wildlife conservation organizations and agencies have spent > $1.0 million Canadian annually on carcass removal (Pat Kehoe, Ducks Unlimited Canada, personal communication). Even though natural resource agencies spend considerable time and money during removal operations, other methods

such as manipulating water levels, and/or vegetation or treating sick birds to recovery have been used (Hunter et al. 1970, Rosen 1971, Locke and Friend 1987, Sandler et al. 1993). However, they have less application and may not be logistically feasible, especially for larger botulism-prone wetlands.

Despite large investments in botulism control, results have been equivocal (Friend 1992). Reed and Rocke (1992) conducted an experimental carcass removal, and found that captive ducks in pens with 12 carcasses/ha were 4.5 times more likely to die of botulism than were birds in pens with no carcasses, suggesting that removal of all carcasses is a useful management technique. Therefore, theoretically, removal efforts should have an impact on reducing mortality. However, under actual field conditions, the effectiveness of removal operations is unknown. Site-specific environmental conditions strongly influence the logistics and success of controlling outbreaks. Molting ducks use habitats with dense concealing emergent vegetation (Gilmer et al. 1977; Oring 1964, 1969). Also, when sick waterfowl become debilitated they seek seclusion in dense cover where they may be missed, some may be removed by predators or scavengers (Samuel 1992), and carcasses may completely decay prior to detection. Efficacy of any removal operation varies due to several factors, including the intensity of the operation, density and size of bird carcasses, and marsh vegetation; Cliplef and Wobeser (1993) evaluated effectiveness of carcass removal on a small Saskatchewan lake and reported that 32.1% of tagged carcasses were recovered. Results of recent investigations are similar (T. Bollinger, Can. Coop. Wildl. Health Ctr., pers. comm.), revealing that thorough carcass recovery is difficult. One carcass can produce thousands of maggots and, depending upon the amount of toxin per maggot, as few as 1-4 maggots can kill a duck (Locke and Friend 1987a, Hubalek and Halouzka 1991) Thus, a single carcass may produce enough toxin-laden maggots to kill hundreds of birds, each of which in turn may produce large numbers of toxin-laden maggots (Wobeser 1997, Wobeser and Bollinger 2002, unpubl).

1.3 THESIS OBJECTIVES AND FORMAT

During botulism outbreaks, the number of dead birds collected can be counted, but typically the mortality rate is not estimated, because the number of wild birds "at risk" is unknown. An implicit management assumption is that carcasses bearing toxic maggots can be reduced to a sufficiently low density that ducks are unlikely to encounter maggots, thereby reducing overall mortality. Therefore, the main objectives of this study were to obtain survival estimates for ducks during botulism outbreaks and to evaluate whether carcass removal operations increase the survival of ducks. The third main goal was to relate survival probability to variation in carcass density, or the number of maggot-laden carcasses. Despite the importance of acquiring reliable information about effectiveness of botulism management, and relationships between survival and carcass density, no similar field study has ever been conducted. This thesis is organized in four main chapters. The present chapter (Chapter 1) provides some background on the avian botulism disease cycle and management techniques and thesis objectives. Chapter 2 provides information on study areas and reports survival rates for mallards exposed to botulism outbreaks. The main objectives of Chapter 2 are to compare estimated survival on wetlands where field removal operations were conducted versus survival on wetlands where no removal operations were attempted. Causes of mortality are reported based on necropsy results. Survival data were further analyzed to explain sources of variation in survival of marked mallards.

Chapter 3 describes carcass density within a dead bird's location compared to that of a randomly chosen live bird. As noted earlier, a major uncertainty about removal of carcasses on wetlands is whether this lowers carcass availability to a density at which duck mortality is substantially reduced. This chapter identifies carcass density thresholds which need to be attained to potentially reduce mortality of uninfected birds on our study wetlands. These estimates are compared to other published estimates. Chapter 4 is a synthesis of main conclusions presented in the thesis and a discussion of the effectiveness of carcass removal operations and duck mortality that occurs during botulism outbreaks. There, I also provide recommendations for future botulism research.

CHAPTER 2. SURVIVAL OF RADIO-MARKED MALLARDS IN RELATION TO MANAGEMENT OF AVIAN BOTULISM

2.1 INTRODUCTION

The prevalence and magnitude of death from avian botulism is poorly understood and may be (often) underestimated, and management to control losses is expensive and time demanding, which led me to estimate survival rates and evaluate effectiveness of carcass removal. Mortality from botulism has been difficult to assess because of complications related to the spatial and temporal variability of botulism outbreaks, the logistic difficulty of studying highly mobile waterfowl populations, and the potentially confounding influences of predation and scavenging on detecting disease-related mortality (Samuel 1992). Regardless, estimating mortality rates from avian botulism is an important step towards improving biological knowledge of this potential threat to waterfowl populations. As waterfowl habitat continues to be degraded or lost, and waterfowl will likely live in a progressively more altered environment in the future, management for all factors, including disease, must account for this inevitability (Wobeser 1997).

My main objective was to evaluate survival of wild molting mallards (*Anas platyrhynchos*) marked with radio-transmitters during botulism outbreaks in prairie Canada to provide a known sample population of "ducks at risk". I estimated survival rates for mallards on each wetland, compared survival among wetlands with surveillance and carcass removal (hereafter, removal lakes) and wetlands where carcasses were not collected (hereafter, non-removal lakes), and determined likely cause(s) of mortality.

2.2 STUDY AREA AND METHODS

2.2.1 Study areas

Study lakes were chosen each year on the basis of history of recent recurrent botulism outbreaks, size of the lake, water-levels in mid-June (to reduce impacts of drought), and opportunity to maximize management agency cooperation and assistance (airboats, surveillance and removal operations) from provincial agencies, the Canadian Wildlife Service, and Ducks Unlimited Canada. In 1999, work was conducted on two large lakes and two small lakes with confirmed botulism outbreaks (Table 2.1). Within each lake size category one was managed (removal of carcasses), while at the other no carcass removal occurred (control). In 2000 and 2001, work focused on smaller lakes to enhance carcass removal (Table 2.1). Three wetlands were used in two years, 2000 and 2001, and treatments were crossed over.

All wetlands were shallow (maximum depth ≤ 2.5 m), lakes with areas of dense emergent vegetation utilized by molting ducks. Wetlands had structures to control water-levels during wet years, but could be completely dry during periods of drought. Each lake had extensive stands of emergent vegetation composed of bulrush (*Scirpus* spp.), cattail (*Typha* spp.) and whitetop rivergrass (*Scholochloa festucacea*)(specific to Whitewater Lake) which ranged from extremely thick to sparse.

2.2.2 Carcass removal operations

On carcass removal wetlands, surveillance began in early to mid June. Areas of open water and vegetation were searched using airboats, whereas shorelines were searched on foot or with all-terrain-vehicles. To increase effectiveness of carcass removal, clean-up and research crews coordinated efforts. The removal operation at Whitewater Lake was delayed but was more intense than previous operations had been in terms of both man-power and the number of boats. Wetlands were divided into workable sectors and Global Positioning System (GPS) technology was available to aid removal crews in better organizing wetland coverage.

Table 2.1. Characteristics of eleven lakes where botulism research was conducted in prairie Canada, 1999-2001.

Year/ Lake	Latitude	Longitude	Area (ha)	Carcass Removal Dates[a]	Boat Hours[b] (Boat hours/ha)	Carcasses Removed (Ducks)	Carcass Removal Cost[c]	Cost/ Carcass(Duck)	Cost/Hectare[d]
1999									
Whitewater, Manit.	49°15':26'	100°19':29'	58,490	12 May-16 Sept.	1170(0.02)	15512(9950)	$125,442.17	$8.09($12.61)	$2.14
Old Wives, Sask.	50°06'	106°00'	79,040	None	NA[e]	0	NA	NA	NA
Eyebrow, Sask.	50°55'	106°08'	850	None	NA	0	NA	NA	NA
2000									
Kettlehut, Sask.	50°39'	106°30'	416	None	NA	0	NA	NA	NA
Paysen, Sask.	50°43'	106°45'	440	6 June-28 Aug.	461(1.05)	2928(1428)	$52,170.37	$17.82($36.53)	$118.57
Frank, Alta.	50°34'	113°43'	601	31 July-25 Aug.	228(0.38)	2351(1495)	$60,000	$25.52($40.13)	$99.83
Crane, Sask.	50°05'	109°05'	950	None	NA	0	NA	NA	NA
2001									
Kettlehut, Sask.			416	8 June-23 Aug.	192(0.46)	87(12)	$36,356.46	$417.89($3029.71)	$87.40
Paysen, Sask.			440	None	NA	0	NA	NA	NA
Frank, Alta.			601	None	NA	0	NA	NA	NA
Chaplin, Sask.	50°22'	106°36'	923	19 June-23 Aug.	215(0.23)	242(38)	$48,272.88	$199.47($1270.34)	$52.30

[a] Removal dates include the period from initial surveillance to the last day of the removal operation.

[b] The number of hours removal crews spent on the water searching for carcasses. These crews usually consisted of one driver and one "picker". Does not include numerous hours spent off the water with duties such as equipment maintenance and repairs.

[c] In Canadian dollars excludes capital cost of equipment, but does include total costs associated with surveillance and removal operations that involves equipment maintenance, repairs, rental, staff wages, housing, staff expenses, and vehicle expenses. Dollar amounts are taken from the removal coordinators' reports. The Whitewater report was prepared by Darcy Pisiak, Manitoba Department of Natural Resources, reports of removals in Saskatchewan were prepared by Steve Stire, South Saskatchewan Field Office of Ducks Unlimited Canada, Regina, SK, and a report on Frank Lake came from Dave Kay, Ducks Unlimited, Brooks, Alberata.

[d] Cost of the removal operation per number of wetland hectares.

[e] Not available because no removals occurred at those locatons.

2.2.3 Trapping and radio-marking mallards

Adult mallards in pre-molt and molt (emerging primaries <10 mm long), were captured and radio-marked prior to and during outbreaks on each wetland. I radio-marked mallards in pre-molt if outer primaries on one wing pulled out easily by thumb and index finger. This was done with the assumption that the birds were going to molt on the lake and to insure an adequate sample of radio-marked birds. The assumption that birds would molt on the lake was probably reasonable because large numbers of molting mallards occurred on all study lakes. From 1 July to the second week of August bait traps were placed simultaneously at several locations on each lake to capture molting male and female mallards; a few were captured by drive-trapping. I attempted to balance the sex ratio of radio-marked birds at each lake, but this was not always feasible given the need to meet the early wing molt criteria and to obtain adequate sample sizes (Table 2.2). Visibly healthy mallards were weighed, banded, and equipped with a back-mounted, temperature-sensitive radio transmitter (172-174 MHz) using standard techniques (Mauser and Jarvis 1991) under local anesthetic (1.2 ml of marcaine), and released. A small external thermometer encased in flexible plastic tubing ran along the underside of the stainless steel anchor. The transmitter's signal pulsed more frequently when triggered by a drop (≥4 °C) in the bird's body temperature, indicating death. Transmitters were designed with a normal signal detection range of ~4 km, ~2.5 km when submerged, and a life span of ~42 days, while retaining favorable features of small size and light weight (12 g). If a marked mallard died in July during banding, the transmitter was re-used if sufficient life-span remained. Holding time for attachment of transmitters was minimized to reduce stress on the birds (Cox and Afton 1998). Methods were approved by the University of Saskatchewan's Committee on Animal Care (Protocol 19980040) on behalf of the Canadian Council of Animal Care.

2.2.4 Tracking the birds

Survival was determined by tracking mallards daily (04:00 - 09:00 CST), sometimes more frequently, for 30 days after release. Bird locations and status (dead or alive) were recorded each morning by tracking with a receiver linked to truck or tower-mounted

antennas. If the transmitter pulse rate had increased, indicating a bird was dead, the carcass was retrieved as quickly as possible with the aid of a hand-held tracking system from an air boat. The location of each dead bird was determined with a GPS (Garmin 12™) unit and the carcass frozen for necropsy.

2.2.5 Necropsies

Necropsies were performed to verify cause of death. Dead birds were included in the survival analysis if they died from botulism or other natural cause such as predation. Botulism toxin causes no visible lesions; intoxicated birds often die from drowning or eventual organ failure. Birds were diagnosed as having died of botulism if 1) botulism was diagnosed in other waterfowl on the lake using serum and mouse bioassay and 2) no other diseases or lesions were identified at necropsy. In some cases carcasses were scavenged and diagnoses of botulism was made if there was no indication of predation (i.e. no hemorrhage) and if the nasopharynx contained leeches indicating the bird was debilitated prior to death. In one case a paralyzed radio-marked mallard was found and serum collected from this duck tested positive for type C toxin. Type C toxin was detected in heart blood and in maggots collected from the esophagus of some ducks. However, this was not used to confirm botulism as maggots were found in only a few ducks, heart blood was frequently of insufficient volume for tests and there was post-mortem growth of bacteria which may result in toxin production.

2.2.6 Statistical analysis

Survival of radio-marked mallards was estimated using known fate models in Program MARK (White and Burnham 1999). The status of most marked mallards was determined each day. However, for logistical reasons, the status of a few mallards was not determined for 2 - 3 days during the 30 day tracking period in 1999 (4 at Whitewater, 2 at Old Wives, 2 at Eyebrow Lakes) and in 2000 (1 at Crane, 3 at Paysen, and 1 at Frank lakes). Each bird was later detected alive so we considered that birds had been present on the lakes yet temporarily undetectable (13/335, 3.9%). Only birds that were reliably tracked for 30 days were included in the survival analyses. Radio-marked

birds that were lost and not recovered as a result of radio failure or other reasons were therefore not included in these analyses. Excluded birds were distributed more-or-less equally among lakes, and the probability of being excluded was unrelated to sex, mass, or date of capture (D. Evelsizer, unpublished).

I assumed recapture probability was 1.0 for all marked mallards included in survival analyses (Lebreton et al. 1993). Survival for 30 days post release was calculated using Kaplan-Meier (Kaplan and Meier 1958) procedure with staggered entry of birds (Pollock et. al 1989). We assumed no effect of radios on survival and that death of each mallard was independent of the fate of others. No goodness of fit checking for over-dispersion was possible because candidate models included individual covariates (White et al. 1999).

The same general *a priori* global starting model was fit to the data each year. Years were analyzed separately for reasons explained below. Lake, sex, body mass, and capture date were included as individual covariates in the general starting model {S (lake+date+sex+mass)}. Survival was initially modeled in relation to "lake" to determine if we could detect lake-specific differences in survival. Sex was used because we radio-marked both male and female mallards (Table 2.2). Sex-specific diets potentially could produce differing survival rates (Rocke and Brand 1994). We also tested if survival was related to body mass, independent of possible sex-related differences. Capture date (days since 1 January) was included to determine whether survival varied through the radio-marking period. I also considered models that included two-way interactions among covariates, but these models received comparatively little support. I constrained time variation as being constant over the 30-day encounter period, because preliminary analyses had indicated that temporal variation in survival rates during this period was minimal.

The most parsimonious (likely) model(s), one with the lowest AICc (Akaike's Information Criterion with an adjustment for sample size) value, was systematically selected from a set of candidate models after removing and re-entering individual covariates. Model weight, beta estimates of individual covariate(s) and difference in AICc values between models (ΔAICc) were considered when comparing

Table 2.2. Body mass (g), and date of radio-marking for male and female mallards on eleven lakes, 1999-2001. Shown are sample size (N), mean ($\bar{x}$) and standard deviation (SD).

	Males					Females				
Year		Body mass		Capture date			Body mass		Capture date	
Lake	N	$\bar{x}$	SD	$\bar{x}$	SD	N	$\bar{x}$	SD	$\bar{x}$	SD
1999										
Whitewater, Manit.	4	1224	136	1 August	3	38	1077	93	28 July	4
Old Wives, Sask.	25	1152	98	30 July	3	4	1006	123	31 July	6
Eyebrow, Sask.	16	1301	106	28 July	3	21	1089	107	30 July	4
2000										
Crane, Sask.	22	1372	127	15 July	3	14	1090	100	23 July	8
Frank, Alta.	8	1179	102	29 July	2	21	1012	77	31 July	4
Kettlehut, Sask.	21	1246	117	19 July	5	4	1106	32	17 July	2
Paysen, Sask.	29	1239	98	18 July	5	1	1175	-	4 August	-

2001										
Kettlehut, Sask.	17	1265	90	18 July	3	9	1062	67	18 July	5
Paysen, Sask.	21	1346	96	16 July	6	6	1161	75	20 July	9
Frank, Alta.	17	1218	136	12 July	10	12	1030	79	22 July	7
Chaplin, Sask.	16	1285	147	11 July	5	9	1102	149	16 July	8

among best fit models (ΔAICc $\leq$ 2.0). The design matrix was manipulated with addition of individual covariates, and parameter estimation was based on a logit-link function to model survival linearly. Therefore, for 1999 and 2000, I report 30 day survival probabilities of flightless mallards exposed to botulism outbreaks derived by model averaging to reduce model selection uncertainty (Burnham and Anderson 1998). With this method best fit models with the highest AICc weight contribute most to the average daily survival rate (DSR). Model averaged daily survival rate $(DSR)^{30}$ was used to estimate 30-day survival probability. Averaged standard error and DSR were used in the delta method (Seber 1982) to calculate 95% confidence intervals reported with survival probabilities.

Separate analyses were performed to estimate survival using (1) birds dying from all natural causes such as predation and disease versus (2) those dying only from botulism. The same set of candidate models was used in both analyses and each set of 30-day survival estimates derived from model averaging.

2.3 RESULTS

2.3.1 Necropsies

Of 418 radios deployed on mallards during 1999-2001, 83 (20%) failed during the tracking period. Therefore, the fate of these mallards was unknown and they were removed from survival analyses. Of the remaining 335 birds that were reliably tracked, necropsies were performed on dead birds that were recovered. All but 2 of these carcasses were sent to the CCWHC to verify cause of death. Necropsies revealed that a few birds died from human induced reasons, including 2 mallards that died of myopathy or stress from handling, 2 died of an infection associated with the transmitter anchor site, and 1 died when it was recaptured in a bait trap. Because these birds died unnaturally they were also excluded from survival analyses. Thus, 330 mallards were used to model survival of molting birds that died of natural causes. Necropsies reported 123 (37%) botulism related deaths, 13 (4%) died of predation, 1 of other diseases, and 1 of an unknown cause.

2.3.2 Survival analyses for 1999

A total of 118 molting mallards was radio-marked in 1999 (Table 2.2). None of seven marked birds from Kimiwan Lake were used in data analyses because one died from predation, and two moved to a neighboring wetland; a sample of four birds was too small to reliably estimate survival. Of 111 marked birds from the remaining three sites, 12 were excluded because the transmitter signal was lost, so 98 birds were included in the survival analysis. Lost signals could have been a result of birds that regained flight and left the wetland during the last few days of the tracking period, or the transmitter was shed or failed. Necropsy results verified all but two birds died from botulism in 1999, both from Whitewater Lake, where one died from predation and the other from other diseases. Another bird was removed because necropsy results suggested death from handling stress. Two carcasses were lost; but death from botulism was probable based on data recorded about the appearance and location of the carcasses when they were recovered in the field so they were included in survival analyses.

Model results revealed wide variation in survival of radio-marked mallards across lakes (Table 2.3). At Whitewater Lake, a removal site, 30-day survival probability of molting mallards was low, similar to Old Wives Lake, which was a non-removal wetland (Table 2.4). Survival probability at Eyebrow Lake, also a non-removal wetland, was higher than at Whitewater Lake. When I then added two birds that died from predation or diseases other than botulism, 30-day survival estimates slightly increased for Old Wives and Eyebrow lakes (Table 2.4).

The top 8 models which included possible lake differences in survival explained 99.9% of the variation in survival based on AICc weight (Table 2.3). Comparison of top four models showed that lake and day of radio-marking in relation to peak botulism mortality had the most influence on survival. Peak botulism mortality occurred around 18 August at Whitewater Lake, 11 August at Old Wives Lake, and 10 August at Eyebrow Lake (Trent Bollinger, CCWHC, pers. comm.). The top model considering lake and day radio-marked and sex {S (lake+date+sex)} was the most parsimonious given the data and models in the candidate set was 2.7 times better supported than the

Table 2.3. Set of candidate known-fate models explaining variation in survival (to 30-days post-release) of radio-marked mallards during botulism outbreaks, 1999-2000. AICc is Akaike's Information Criterion adjusted for sample size.

Year/ Model	AICc	ΔAICc[a]	AICc Weight[b]	K[c]
1999				
{S(lake+date+sex)}	466.853	0.00	0.451	5
{S(lake+date+sex+mass)}[d]	468.855	2.00	0.166	6
{Slake+date)}	469.139	2.29	0.144	4
{S(lake+date+mass)}	470.046	3.19	0.091	5
{S(.)}	486.530	19.68	0.000	1
2000				
{S(lake+mass)}	528.710	0.00	0.282	5
{S(lake)}	529.815	1.11	0.162	4
{S(lake+sex+mass)}	530.615	1.90	0.109	6
{S(lake+date+mass)}	530.713	2.00	0.104	6
{S(lake+sex)}	530.918	2.21	0.093	5
{S(lake+date)}	531.231	2.52	0.080	5
{S(mass)}	531.676	2.97	0.064	2
{S(lake+date+sex+mass)}[d]	532.592	3.88	0.040	7
{S(.)}	541.927	13.22	0.000	1

[a] Difference between current model and best approximating model.

[b] Weight of evidence in favor of model, relative to those in candidate list, weights sum to 1.0.

[c] Number of parameters. Note: analyses involved 3 lakes in 1999 and 4 lakes in 2000. Date and mass were treated as continuous predictors.

[d] Global starting model.

Table 2.4. Estimated survival (to 30-days post-release) of molting mallards radio-marked on eleven lakes on the Canadian prairies, 1999-2001. Also shown is whether carcass removal operations were conducted or there was no attempt to remove carcasses, and the number of birds (N) included in survival analyses comparing all causes of natural mortality versus only avian botulism mortality.

Year		Survival with all causes of mortality [a]		Survival with botulism mortality only[b]	
Lake	Treatment	N	Survival (95%CI)	N	Survival (95%CI)
1999					
Whitewater	Removal	41	0.046 (0-0.112)	39	0.046 (0-0.115)
Old Wives	Non-Removal	24	0.084 (0-0.271)	24	0.043 (0-0.174)
Eyebrow	Non-Removal	33	0.421 (0.168-0.675)	33	0.386 (0.129-0.643)
2000					
Kettlehut	Non-Removal	25	0.668 (0.515-0.822)	23	0.700 (0.520-0.880)
Paysen	Removal	29	0.667 (0.522-0.812)	27	0.705 (0.544-0.865)
Frank	Removal	26	0.491 (0.270-0.712)	25	0.351 (0.099-0.603)
Crane	Non-Removal	37	0.604 (0.448-0.759)	36	0.543 (0.363-0.723)

2001					
Kettlehut	Removal	28	0.929 (0.756-0.982)	26	1.00 (1.00-1.00)
Paysen	Non-Removal	31	0.774 (0.597-0.888)	28	0.886 (0.701-0.963)
Frank	Non-Removal	30	0.829 (0.649-0.927)	28	0.860 (0.681-0.946)
Chaplin	Removal	26	0.962 (0.773-0.995)	26	0.962 (0.773-0.995)

[a] All causes of death include avian botulism, predation, other underlying diseases and unknown natural causes, and the respective 30-day survival estimates.

[b] Sample of birds factoring in only cause of death from avian botulism, and the respective 30-day survival estimates.

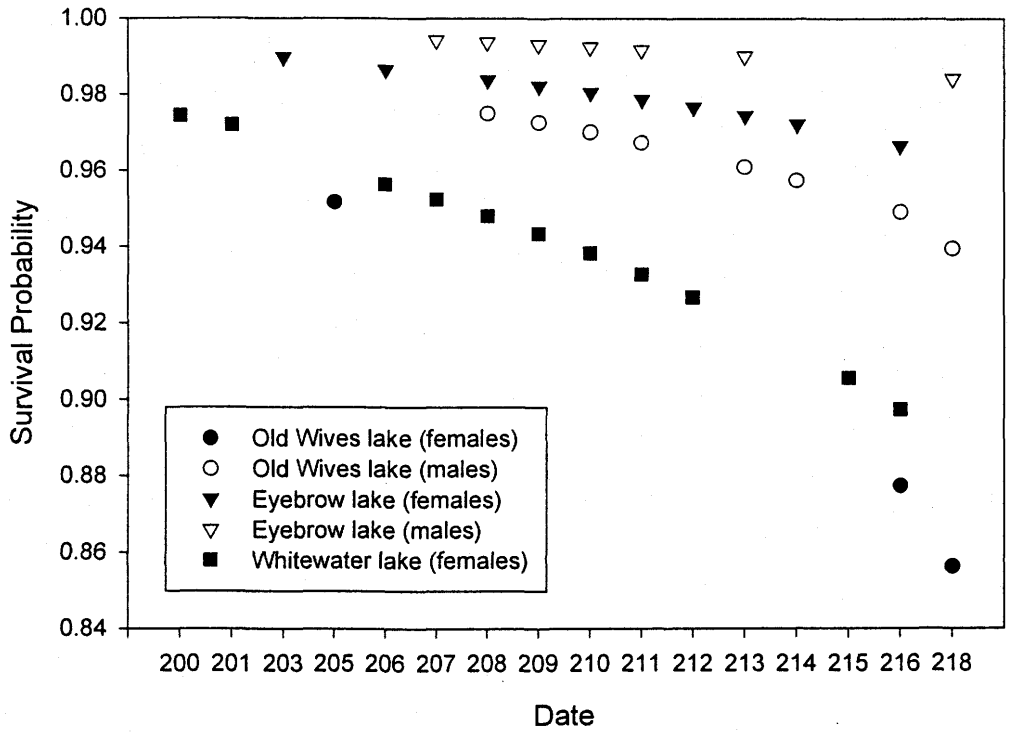

Figure 2.1. Relationship between daily survival probability and date of capture for molting male and female mallards radio-tracked on three botulism outbreak lakes (1999), based on reconstituted parameter estimates from model {S (lake+date+sex)} (Table 2.3). Date is days since 1 January (day 200 is 19 July).

global model {S (lake+date+sex+mass)} (ΔAICc = 2.0)(Table 2.3). Survival was negatively related to capture date (β = -9.229, SE = 3.874, 95% CI = -16.821 to -1.636). The shape of this function was plotted for the three lakes (Fig. 2.1). The function relating survival to capture date and sex was derived from logit(*S*) values of individual birds produced in Program MARK (Cooch 1999). Daily survival probability decreased linearly with later capture date at all three lakes, and females had a lower survival than males (95% CI = -1.903 to -0.021). The top model {S (lake+date+sex)} was substantially more likely than a model without sex {S (lake+date)}(ΔAICc = 2.29). Overall survival varied among lakes, with date radio-marked being more influential than a bird's sex and mass.

2.3.3 Survival analyses for 2000

A total of 160 molting mallards was radio-marked in 2000, but unlike 1999, transmitter failure accounted for 24% of the total sample. Surrounding uplands were scanned with the truck or tower mounted antennas in case predators had carried a carcass containing the transmitter away from the wetland. Transmitter loss or predation was ruled out because (small) lakes were thoroughly searched with hand held antennas from an airboat for 3 to 4 days after the bird was lost. After removing these birds, 117 mallards were used in survival analyses. Results of necropsies verified most birds died from botulism. Five birds died from predation and 1 unknown cause of death. Two birds likely died as a result of infection and 1 from handling stress, and were omitted. Thirty-nine of 117 radio-marked mallards were adult females and 78 were males (Table 2.2).

Survival to 30 days was estimated using model averaged DSR for mallards from four lakes. Survival estimates varied among lakes in 2000, similar to 1999 results. Survival probability was lowest at Frank Lake, a removal site, followed by Crane Lake, a non-removal site (Table 2.4). These two lakes were medium size lakes, being larger than Paysen and Kettlehut lakes (Table 2.1), and survival was generally lower on smaller lakes. There was little difference in the survival estimates when adding in birds that died from predation or unknown natural causes of death (Table 2.4). The 95%

confidence intervals overlapped within a few percentage points with the greatest difference occurring at Frank Lake.

Lake differences were again most influential, with top 6 models explaining 83% of variation in survival (Table 2.3). The top model, {S(lake+mass)}, was 1.7 times better supported than model {S(lake)}. Survival increased with mass (95% CI = -2.19, 39.15) at all four lakes (Fig. 2.2). There was low precision (β = 0.067, 95% CI = -0.336, 0.469) to detect sex-related differences in survival in model {S(lake+sex+mass)}, possibly resulting from uneven sex ratios across lakes (Table 2.2). Capture date (95% CI = -0.067, 0.643) was in the top 5 models {S (lake+date+mass)}, but was less influential than lake and mass (ΔAICc = 2.0).

2.3.4 Survival analyses for 2001

A total of 147 molting mallards was radio-marked in 2001(Table 2.4), with 109 used in survival analyses. Radios were deployed on molting mallards beginning 30 June 2001 with most birds being radio-marked from 10 July to 2 August. Seven radio-marked mallards were killed by predators at Kettlehut, Frank, and Paysen lakes. Survival of radio-marked mallards was slightly lower when birds killed by predators and botulism were considered. Because there was much less variation in survival in 2001, survival estimates were obtained from model {S (lake)}. Kettlehut was a removal lake and had no detectable botulism. Chaplin, the other removal lake, had low levels of botulism. Frank and Paysen, were lakes with no removal, also had low levels of detectable botulism (Table 2.4). Survival did not vary sufficiently to consider effects of date, sex, or mass.

There were fewer and less severe botulism outbreaks across prairie Canada in 2001. This pattern was reflected at our research sites. On both lakes designated as removal sites, botulism was not detected (Kettlehut Lake) or did not develop into a severe botulism outbreak (Chaplin Lake).

Removal operations started at Chaplin Lake on 12 July and ended 23 August 2001. Although 242 carcasses were recovered (Table 2.1), most (133) were found along the shore and were dried out skeletons believed to be from the die-off the previous year

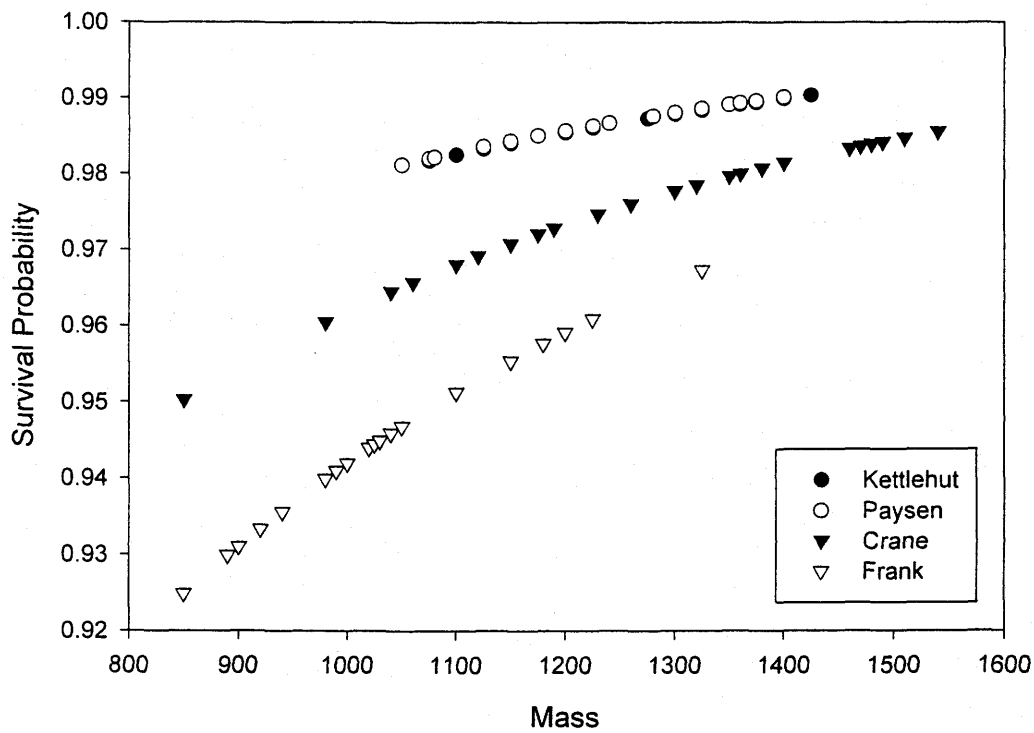

Figure 2.2. Relationship between daily survival probability and body mass (g) for molting mallards (sexes combined) radio-marked at four botulism outbreak lakes (2000), based on reconstituted parameter estimates from model {S (lake + mass)} (Table 2.3).

(Steve Stire, DU-Canada, personal communication). Only 7 mallard and 7 American coot (*Fulica americana*) carcasses were identified. Removal started at Kettlehut Lake on 16 July and ended 23 August 2001. Of 87 carcasses recovered, 59% were hatch-year flightless American coots and 26% were hatch-year flightless eared grebes (*Podiceps nigricollis*). Furthermore, 17% of carcasses were collected on 2 August following a hail storm on 1 August. It is important to note, however, that even in a year when there appeared to be little botulism-related mortality, there was a 14% and 11.4% mortality rate at Frank and Paysen lakes, respectively (Table 2.4). Also, mortality that occurred on these two lakes would have probably gone undetected or would have not been considered sufficient to warrant a removal operation under a traditional surveillance regime.

2.4 DISCUSSION

2.4.1 Survival

This study reports the first estimates of survival rates of wild birds exposed to avian botulism. Radio-tracking of molting adult mallards was employed successfully in 1999 on all sites. Clearly, however, the removal effort at Whitewater Lake did not enhance survival of radio-marked mallards (Table 2.4). The highest mortality rates occurred at Whitewater and Old Wives lakes where estimates were similar. In hindsight this is not surprising with consideration of the size and logistics of dealing with removal on a large lake. The original study design called for intense, early removal on both treatment lakes, a plan which was executed at Kimiwan but not at Whitewater. Surveillance began in May at Whitewater Lake (Table 2.1), but a full-scale relatively intense carcass retrieval operation did not begin until after large numbers of carcasses were already present.

Wetland size, the amount of emergent vegetation, or other factors may be important considerations when comparing the impact of removal efforts, and learning whether intense surveillance and removal could reduce duck mortality was the primary objective of the study (Table 2.1). After 1999, lake-size was scaled down, and removal efforts

were intensified. In 2000, therefore, research was conducted on four small lakes. Survival variation across lakes was most influential, with lowest survival recorded at Frank Lake, a removal site and Crane Lake a non-removal site (Table 2.4). Paysen (removal) and Kettlehut (non-removal), located in the Thunder Creek drainage system, were similar in size; mallards on these two small lakes generally had higher survival than Frank and Crane lakes. The two removal lakes (Frank and Paysen) were relatively small, had less emergent vegetation, and good access (Table 2.1). Removal operations were delayed slightly at Frank Lake but an intense removal response was still executed quickly. The removal at Paysen Lake made smooth progress, although it was recommended that intense removals begin about two weeks before carcasses build up to allow training of competent airboat operators. Removal did not consistently enhance survival of radio-marked birds in 2000, as judged by the low survival on Frank Lake, and small difference in survival rates between Paysen and Kettlehut Lakes. Furthermore, removal operations were expensive and time-demanding, even on smaller lakes (Table 2.1). Overall, survival variation among lakes seemed to be related to severity of the botulism outbreaks at each wetland.

Paysen Lake (440 ha) had a very intense removal effort during the botulism outbreak in 2000. On most days removal crews had 3 airboats and crews running on the lake (Steve Stire, pers. comm.), achieving 1.05 boat hours per hectare (Table 2.1), and searching the entire lake every 2 days. I used these data to extrapolate estimated effort needed to achieve removal efforts of equal proportions at Whitewater and Old Wives Lakes. Whitewater Lake is 58,490 ha, 133 times larger than Paysen; thus, 399 boats and 798 people (2/boat) would be required to achieve 1.05 boat hours per hectare. The estimated cost of a removal of this intensity at Whitewater Lake would be $6.9 million. Using the same calculations for Old Wives Lake (79,040 ha), 540 airboats and a minimum of 1080 people would be required, with an estimated cost of $9.4 million dollars. Even with the effort at Paysen Lake in 2000, there was still a 30% mortality rate of radio-marked mallards (Table 2.4).

Effort on the two removal lakes in 2001 was considered very intense. Removal crews were trained and equipment prepared in early July, much earlier than in traditional

responses to outbreaks. However, the cost of executing a removal of this sort was expensive (Table 2.1). The cost/duck removed was higher in 2001 than any other year, because there were fewer carcasses recovered relative to effort expended. When an outbreak is more severe, and clean-up crews remove large numbers of dead birds, cost efficiency (cost per duck removed) improves. Perhaps a better measure of cost effectiveness would be cost per duck saved. Although impossible to evaluate thoroughly, my results suggest that survival could not be increased by carcass removal and, therefore, the number of ducks saved was not related to management effort.

The relationship is shown that daily survival probabilities decreased for molting male and female mallards radio-marked later in the capture period during mid July to mid August, 1999 (Fig. 2.1). The two large lakes both had relatively severe botulism outbreaks, with increased mortality of ducks occurring in late July through August. All wild mallards captured and radio-marked were in pre-molt or in early molt stages and their flightless period coincided with peak botulism mortality occurring at the lakes.

Too few males were radio-marked at Whitewater Lake to detect a sex-related difference in survival. Female survival was lower than that of males at Old Wives and Eyebrow lakes, which contrasts to Rocke and Brand's (1994) finding that captive male mallards contracted botulism at a higher rate than females. This may be because wild females were in poorer condition than males when they began molt and therefore were more vulnerable to botulism toxin. Many female ducks molt later in the summer than males, typically when botulism outbreaks reach peak mortality levels in a wetland.

The relationship of increased survival with increased mass was detected for the four lakes in 2000 (Fig.2.2). Survival in relation to bird features of sex and mass may reflect the intensity of botulism outbreaks at these wetlands. In both years survival variation was best explained by lake differences (Table 2.3). Intensity of the outbreaks may be a function of spore density, bacteria, bacteriophage, substrates, transfer of toxin, bird usage or other factors working together in a given year and wetland (Wobeser and Bollinger, 2002 unpubl). The outbreaks that occurred on these lakes were less severe than the outbreaks at Whitewater and Old Wives lakes in 1999. A mass and survival relationship was not detected in 1999 and survival variation was better explained in

relation to timing of radio-marking molting mallards in relation to peak mortality. In 2000, when outbreaks were less severe, the survival probability increased for heavier mallards with no sex-related differences, suggesting an interaction between mass and toxicity of toxin. There may be variation in toxin toxicity produced within vertebrate carcasses at different wetlands.

In the absence of botulism mortality, there was little mortality from predation or other natural causes of radio-marked mallards (Tables 2.4). At the smaller lakes in 2000 and 2001, predation had slightly more impact on survival. This could be attributable to predator access within and around the smaller wetlands. Mammalian predators observed in the vicinity of the lakes included coyotes (*Canis latrans*), foxes (*Vulpes vulpes*) and mink (*Mustela vison*). Avian predators observed were northern harriers (*Circus cyaneus*) and Swainson's hawks (*Buteo jamaicensis*) primarily.

2.4.2 Capture and radio-marking biases

Because I used bait traps to capture most mallards, there may be a condition bias in the sample. Condition biases in waterfowl trapped for banding have not been documented, but banded mallards, in poor condition (indexed by mass/ wing length) were more likely to be recovered than were good-condition birds during the ensuing hunting season (Hepp et al. 1986, Dufour et al. 1993). Conroy et al. (1989) showed that female American black ducks (*Anas rubripes*) with below-median body masses were at greater risk of hunting mortality than were those with above median body mass. I found that, in general, survival was directly or indirectly (via sex effects) related to body mass. If my radio-marked samples were composed of mostly light-weight, poor condition mallards, my survival estimates could be biased low. I have no way of rigorously addressing this problem.

Another important assumption is that using radio-transmitters does not bias survival estimates. Early studies recorded negative behavioral effects of back-mounted harness-style radio-packages on dabbling ducks (Greenwood and Sargeant 1973, Gilmer et al. 1974), and more recently, studies have shown that backpack style transmitters with harnass attachments had negative affects on reproduction (Rotella et al. 1993, Dzus and

Clark 1996). Pietz et al. (1993) found that wild female mallards with harness-style backpacks fed less, and rested and preened more than unmarked birds. Bowman and Longcore (1989) used harness-style transmitters on molting black ducks (*Anas rubripes*) during the 29 day flightless period, had a survival estimate of 0.874 (95% CI = 0.675 - 0.987).

To avoid some of the pitfalls associated with harness attachment methods, I used a technique (backpack with prong and sutures) that was developed for attaching transmitters to newly hatched ducklings (Mauser and Jarvis 1991). Paquette et al. (1997) used a similar anchored backpack transmitter on mallards. They detected a trend that survival was lower but was not significant overall; and noted this was during the reproductive period when female mallard survival overall is lower than during the post-breeding season (Cowardin et al. 1985, Losito et al. 1995).

2.4.3 Study design considerations

I evaluated management effectiveness on wetlands where carcasses were removed and on those not subject to management. Strict adherence to normal experimental protocols (i.e., random allocation of treatment/control, replication, cross-over, data independence) was not possible. No two wetlands or outbreaks are similar in all respects (Wobeser et al. 1987, Sandler et al. 1993). The best approach would have been to sub-divide wetlands with histories of botulism and applied different management scenarios to the various sub-divisions so that a randomly selected portion of each wetland served as its own control. However, the movements of radio-marked birds would have compromised this design. For instance, in 1999, radio-marked birds utilized large portions of wetlands, moved across lakes, and were not always confined to relatively small areas.

Based on direct experience in 1999, it was obvious that a removal operation on a lake the size of Whitewater was futile. So, in 2000 and 2001, research only included lakes that were much smaller than Whitewater or Old Wives (Table 2.1), to enhance the chances of finding a positive effect of removal operations. Smaller wetlands had less area to search for carcasses, allowing for more complete, faster coverage, of the lakes.

2.4.4 Conclusions and future recommendations

Necropsies verified that botulism was responsible for 89% of the mortality in radio-marked mallards. The second leading cause of mortality (9%) was predation. Most botulism mortality occurred at Whitewater and Old Wives lakes. Overall, survival rates varied among lakes in all three years (Table 2.4). Model results revealed most of the variation in survival was explained by differences among lakes (Table 2.3). We also detected other sources of variation in survival related to the timing of peak botulism mortality, and bird features of sex and mass (Fig 2.1, Fig. 2.2). Removal operations had no detectable effect in improving bird survival. Generally, differences in survival were dependent on the severity of the botulism outbreaks that occurred at each of the study wetlands, regardless of whether or not removal operations were conducted.

As waterfowl habitat continues to be degraded or lost, management of remaining wetlands becomes increasingly important, especially wetlands that are attractive to molting ducks during the post breeding and molting time in late summer. Work is needed in the future regarding avian botulism, especially in prairie Canada, where the occurrence and prevalence of massive die-offs exist and often go unnoticed or have very little monitoring efforts attempted. Future work is needed to determine the impact of avian botulism outbreaks. Although, this study has determined that removal efforts are futile, and mortality can be alarmingly high especially on large lakes where the most mortality is occurring, there is much opportunity for future research efforts.

Future work could determine whether and under what conditions botulism reduces waterfowl populations. It is suspected that botulism effects local populations around the lakes where die-offs occur, yet it is unknown to what extent these losses affect continental populations. Continued banding operations on botulism and non botulism lakes may help answer this, but estimates of the number of ducks exposed to botulism are required. Furthermore, body mass and other considerations suggest that botulism may have greater impact on northern pintails (*Anas acuta*) than on mallards. Future radio-marking and banding may be useful in understanding disease impacts on pintails.

Other work could focus on establishing a standard protocol for monitoring mortality at botulism outbreak sites across prairie Canada. Since there can be many wetlands with

botulism outbreaks in a given year, critically important wetlands could be identified, and monitored more intensively. Determining which wetlands have the most mortality, and why, would be of utmost importance. If a standard survey of mortality was established and performed over time (years), it could provide a basis for prioritizing management decisions. Spring waterfowl pair counts is one of the largest and most organized bird survey conducted annually in the Canada and the United States. However, relatively little effort is devoted to monitoring duck populations after nesting and to the fall hunting season. For example some wetlands such as Eyebrow Lake, Old Wives Lake, and Whitewater Lake may have consistent annual die-offs. They could potentially be more important to deal with than lakes such as Pakowki, Crane or Kettlehut lakes. Although, with some monitoring, it may be found these lakes may not have consistent die-offs, but when they do, large numbers of ducks are involved and would therefore, be of consideration as well. Establishing a standard survey of estimating annual botulism mortality using a technique such as airboat transects across lakes would provide the opportunity to characterize mortality and/or any mortality patterns occurring over time. Blood samples should be taken to confirm botulism, and verify toxin toxicity among wetlands. Annual and consistent procedures would also provide more information of species composition, sex, and age traits associated with mortality from botulism.

CHAPTER 3. RELATIONSHIPS BETWEEN LOCAL CARCASS DENSITY AND SURVIVAL OF MOLTING MALLARDS DURING AVIAN BOTULISM EPIZOOTICS

3.1 INTRODUCTION

Maggot-laden carcasses are considered to be a source of botulism toxin for waterfowl (Duncan and Jensen 1976); and vertebrates that have died from any cause in a wetland can initiate and perpetuate botulism through a carcass-maggot cycle (Reed and Rocke 1992, Wobeser 1997). The goal of carcass removal operations is to stop the carcass-maggot cycle, and therefore reduce mortality in healthy birds. However, results of carcass removal operations are equivocal; on large lakes, and on smaller lakes. One of the questions that remain is; if removal operations are conducted, do carcass density thresholds exist that, if reached, could rapidly increase survival of wild live birds? Reed and Rocke (1992) found that captive mallards in pens with 12 carcasses/ha were 4.5 times more likely to die of botulism than were birds in pens with no carcasses. Following those results, I predicted that survival would be related to carcass density with wild birds. And if so, does this relationship vary among lakes, similar to the variation in survival probability found in Chapter 2. Variation among lakes was found with toxin toxicity tests from blood serum samples that ranked in the "high" range collected from Whitewater and Old Wives Lakes, but in the low to medium range from other botulism study wetlands (Trent Bollinger, pers. comm.).

Therefore, the main objective was to use a known sample population "birds at risk" of wild radio-marked mallards tracked during botulism outbreaks on various wetlands to compare carcass density variation at the locations of dead and live birds. In this paper, I describe 1) methods for monitoring wild mallards in wetlands, 2) report differences in carcass densities at dead and live bird locations, 3) model survival probability in relation

to carcass density and compare it to a previously published estimate, and 4) identify threshold carcass densities among study wetlands.

3.2 METHODS

3.2.1 Intense carcass investigations

Capture, marking, and daily radio-tracking methods and protocols were described in Chapter 2. Location and status (dead or alive) of each bird were recorded each morning by tracking with a receiver linked to truck or tower-mounted antennas. If the transmitter pulse rate had increased, indicating a bird was dead, the carcass was retrieved as quickly as possible with the aid of a hand-held tracking system from an airboat. The location of each dead bird was determined with a GPS unit and the carcass frozen for necropsy. A 5 cm x 5 cm x 300 cm (2"x 2" x 10') wooden stake was placed where the carcass was found. A search for other carcasses was then completed within a 50 m radius (0.785/ha plot) of (1) the dead mallard's location and on the same day at (2) a randomly-selected, live radio-marked mallard's location. Live radio-marked birds were selected by their respective radio frequency number randomly drawn out of a hat without replacement each day a carcass investigation(s) was conducted. The live bird location was determined by going to its morning location, and then moving slowly to the bird's position using a hand held tracking system from the airboat; this best estimate of the live bird's location was marked with a wooden stake.

At the start of each intense search a tape measure was stretched to mark a 50-m radius in four cardinal directions from the wooden stake. Colored flagging tape was tied to vegetation, or a wooden stake was placed at these points to aid search crews. In water <1 m deep, the search was conducted by crews systematically wading through the area beginning at the center and fanning out in a spiral fashion to ensure an organized search. People were spaced 1 m apart in dense vegetation, and ≥3 m apart in sparse vegetation. Searches were also conducted from an airboat in a systematic pattern. During the search, crews recorded total number of carcasses found, and whenever possible, each

carcass was assigned to species, age, sex, molt status, and the stage of decomposition (Table 3.1).

3.2.2 Characterization of stages of decomposition (SOD)

The standard method utilized by research crews was to classify each carcass to one of six stages of decomposition (SOD) by comparing carcasses to colored photos and SOD descriptions. SOD 1 was a "sick" live bird with some paralysis. SOD 2 was a "freshly dead" carcass with no maggots. SOD 3 was a water sodden carcass in early stages of decay but no maggots, distinguished by green skin when the feathers were pulled back. A SOD 4 carcass had small maggots visible under the feathers. SOD 5 carcasses had larger internal maggots, maggots had penetrated the skin, usually creating obvious "maggot rafts". The last stage was SOD 6, when carcasses were characterized as skeletons, with keel and ribs exposed and some remaining feathers and skin intact but most flesh was eaten, and few remaining maggots. To summarize, SOD 1to 3 were sick birds or carcasses in early stages of decomposition, whereas, SOD 4 to 6 carcasses were in later stages of decay with maggot development.

3.2.3 Statistical analysis

To evaluate the relationship between variables of radio-marked bird locations and survival probability, I employed logistic regression analyses (PROC CATMOD, SAS Institute 1999), specifying bird status (i.e., dead vs. alive) as a binary response variable and lake, carcass density, and date of search as initial explanatory variables. There was insufficient variation in survival in 2001 to include these data. I modeled each of 7 lakes separately for years 1999 and 2000 (Table 3.2). However, retaining "lake" was not well supported in the set of candidate models. So the 7 lakes were pooled into two categories (Table 3.2). A "high risk" group was created for Whitewater and Old Wives lakes because there was 95% mortality of radio-marked mallards at these two sites. The other 5 lakes were grouped as "low risk" based on their lower estimated mortality rates. Pooled lakes were included as the 'categ' variable in the models. Carcass density (carcasses per hectare) was calculated as the number of carcasses found per area

Table 3.1. Carcass densities (carcasses/ha) near dead and live radio-marked mallards, 1999-2000. Shown are number of searches (N), mean carcass density ($\bar{x}$), and carcass density range (Range).

	Dead birds						Live birds					
	All carcasses[a]			Maggoty carcasses[b]			All carcasses[a]			Maggoty carcasses[b]		
Lake	N	$\bar{x}$	Range	N	$\bar{x}$	Range	N	$\bar{x}$	Range	N	$\bar{x}$	Range
Whitewater[d]	30	13	0-39	25	5	0-28	11	2	0-10	8	0	0
Old Wives	13	15	1-51	NA[c]	-	-	13	2	0-9	NA	-	-
Eyebrow	12	15	5-29	7	13	3-28	12	6	0-17	9	4	0-9
Crane	16	11	0-71	16	5	0-11	16	6	0-19	16	4	0-9
Frank[d]	21	10	1-21	21	4	0-9	21	5	0-13	21	2	0-7
Paysen[d]	8	8	0-38	8	4	0-20	8	6	0-24	8	3	0-11
Kettlehut	8	14	0-33	8	8	0-18	8	9	0-42	8	7	0-35

[a] Includes all carcasses found in search plots (SOD 1-6).

[b] Includes only carcasses in stages of decomposition with maggots present (SOD 4-6).

[c] Carcasses found in search plots were not recorded by SOD stage; therefore, data are not available.

[d] Carcass removal was conducted.

Table 3.2. Set of candidate models predicting status ("dead" or "alive") of radio-marked mallards, 1999-2000. AICc is Akaike's Information Criterion adjusted for sample size.

Model	AICc	ΔAICc[b]	AICc Weight[c]	K[d]
categ[a], density, categ*density	233.649	0.00	0.711	4
categ, density, date, categ*density	235.713	2.06	0.253	5
categ, density	240.765	7.12	0.020	3
categ, density, date	242.843	9.19	0.007	4
density	243.073	9.42	0.006	2
density, date	245.039	11.39	0.002	3
lake, density, date, lake*density	247.285	13.64	0.001	11
lake, density	248.173	14.52	0.000	9
lake, density, date, lake*density, density*date[e]	249.001	15.35	0.000	12
null	273.286	39.637	0.000	1

[a] 'Categ' is the lake variable categorized into high risk of mortality or low risk of mortality.

[b] Difference between current model and best approximating model.

[c] Weight of evidence in favor of model, relative to those in candidate list, weights sum to 1.0.

[d] Number of parameters.

[e] Starting model.

searched. Date was days since 1 January. I also tested for effects of interactions involving lake by density, density by date, and "risk category" by density. This involved developing a starting model of the form

$$S_i = \exp(\beta_0 + \beta_1 d_i + \beta_2 \ldots \beta_5) / 1 + \exp(\beta_0 + \beta_1 d_i + \beta_2 \ldots \beta_5) \quad (3.1)$$

where S_i is the probability that individual i is alive, d_i is the carcass density at the location of individual i at the time of the search, and β_0 and $\beta_{1\text{-}5}$ are parameters to be estimated. The essential feature is that the sign and magnitude of $\beta_{1\text{-}5}$ indicate the direction and strength respectively of the relationship between carcass density, lake, date, risk category, and interactions of these variables. Likewise, I obtained maximum-likelihood estimates (MLE) of β_0 and $\beta_{1\text{-}5}$ and used model inference of the null intercept only model. Akaike's Information Criterion (AICc) was calculated using the maximum-likelihood estimate and number of parameters adjusted for sample size (Burnham and Anderson 1998). The most parsimonous (likely) model(s), one with lowest AICc value, was systematically selected from a candidate set after removing and re-entering predictor variables, using the general starting model. Model weight, number of parameters, and difference of AICc values between models (ΔAICc), were considered when comparing the fit of top models ($\Delta\text{AICc} \leq 2.0$). Model weight was calculated to quantify model strength (Burnham and Anderson 1998):

$$W_i = [\exp(-\Delta\text{AICc}/2)] / \Sigma[\exp(-\Delta\text{AICc}/2)] \quad (3.2)$$

Parameter estimation was based on a logit link function to model survival linearly. Therefore, I selected the model that best represented the relationship of the predictor variables for further examination; MLEs were used to derive and plot predicted survival probability in relation carcass density, and risk category.

Separate analyses were performed to evaluate carcass density divided by carcasses with maggots and those without maggots (Table 3.3). Carcasses that had decomposed to the point of having internal maggots are thought to be the greatest threat in transmitting

Table 3.3. Set of candidate models with carcass density separated into stages of decomposition (SOD) to predict mallard survival probability on low risk botulism lakes. The 'maggots' variable is SOD 4 to 6 carcasses with maggots and 'nomag' is carcasses in early stages of decomposition, SOD 1 to 3 without maggots. AICc is Akaike's Information Criterion adjusted for sample size.

Model	AICc	ΔAICc[a]	AICc Weight[b]	K[c]
maggots	167.45	0.00	0.673	3
maggots, nomag	169.59	2.14	0.231	4
nomag	172.03	4.58	0.068	3
lake, maggots	174.50	7.05	0.020	7
lake, maggots, nomag[d]	176.72	9.27	0.007	8
lake	180.20	12.74	0.001	6
lake, nomag	180.60	13.15	0.001	7

[a] Difference between current model and best approximating model.

[b] Weight of evidence in favor of model, relative to those in candidate list, weights sum to 1.0.

[c] Number of parameters.

[d] Starting model.

botulism to other healthy birds (Hunter et al. 1970). In this set of candidate models I separated carcasses with and without maggots to determine effects on survival probability at only the low risk lakes. Although total carcass density was available, carcass SOD was not recorded at Old Wives Lake, therefore that data was not available. Whitewater was determined to be a high risk lake in previous models, so in this set of models I included only the five low risk lakes (Table 3.3). The 'lake' variable was included to test for any residual lake effects among the low risk wetlands.

3.3 RESULTS

3.3.1 Intense carcass searches

Of 197 intense carcass searches conducted at 7 botulism outbreak sites during 2 years of the study, 108 were dead bird locations, and 89 were random, live bird locations (Table 3.1). In 1999, many more birds died and, due to logistical reasons more dead bird than live bird searches were completed. Searches were conducted from 26 July to 30 August 1999, and from 15 July to 27 August 2000. Overall, carcass density (carcasses/ha) was higher at the dead bird locations ($\bar{x}$ = 11.6, SE = 0.986, median = 10, range 0-71) than at random live bird locations ($\bar{x}$ = 5, SE = 0.658, median = 4, range 0-42).

3.3.2 Model selection

Models including individual lakes were not important (ΔAICc $\geq$ 13.64, model weight $\leq$ 0.1%) (Table 3.2). So I pooled lakes into either a high or low risk category. Whitewater and Old Wives lakes were considered "high risk" sites and the other 5 lakes were "low risk". Model results showed a much better fit to the data when pooling the lakes into risk categories (Table 3.2). The top two models, with a risk categ * density interaction, had 96.4% model weight. The top model had 71.1% of model weight and was 2.8 times better supported by data than the second model (with an additional parameter, date) which had 25.3% model weight (ΔAICc = 2.06). The third best model, with risk categ and density and no interaction term, had only 2% support (ΔAICc = 7.12). Therefore, there was strong support for effects of carcass density and "risk category" in survival;

however, the effect of density on survival differed between low and high risk lakes. Therefore, I looked at these relationships in greater detail.

3.3.3 Carcass density models and survival probability

The relationship of carcass density and survival probability was portrayed using the intercept (β_0) and slope ($\beta_1 d_i$) terms taken from logistic regression (Fig. 3.1). For both high and low risk lakes, estimated survival probability decreased with increasing carcass density. However, at high risk lakes, survival decreased most rapidly between 0 and 12 carcasses/ha. At the 5 lower risk lakes survival decreased gradually from 0 to 50 carcasses/ha (Fig. 3.1). Survival probability was 0 at the high risk lakes when carcass density was ≥20, and at the low risk lakes when carcass density ≥65. Survival of radio-marked mallards on high risk lakes was more sensitive to carcass density than on low risk lakes. Radio-marked mallards still had a chance of dying when carcass density was equal to 0 on high and low risk lakes ($S = 0.74$ and $S = 0.66$, respectively). This could occur if birds that died had moved >50 m after becoming intoxicated, into areas of lower carcass density, or the locations of live birds were determined imprecisely.

Carcass density was truncated at 20 carcasses/ha on both high and low risk lakes (Fig. 3.2) because most searches obtained carcass estimates that ranged from 0-20 carcasses/ha. The intercept and slope of these relationships better represented survival probability since carcass densities of <20 occurred most frequently (Fig. 3.2). Also, I found the greatest change in survival probability was at low carcass densities. The rate of decrease in survival probability was more rapid at the high risk lakes especially from 0-12 carcasses/ha than at the low risk lakes.

3.3.4 Maggoty versus non-maggoty carcasses

I further evaluated effects of carcass density on survival by separating carcasses into those in late stages of decomposition (SOD 4 to 6) and those without maggots (SOD 1 to 3). At Whitewater Lake, no live birds had maggoty carcasses within 50-m radius (n = 8), whereas dead birds had a mean of 5.25/ha (n = 25, range = 0-28) (Table 3.1). SOD descriptions of carcasses were not available for Old Wives Lake. At low risk

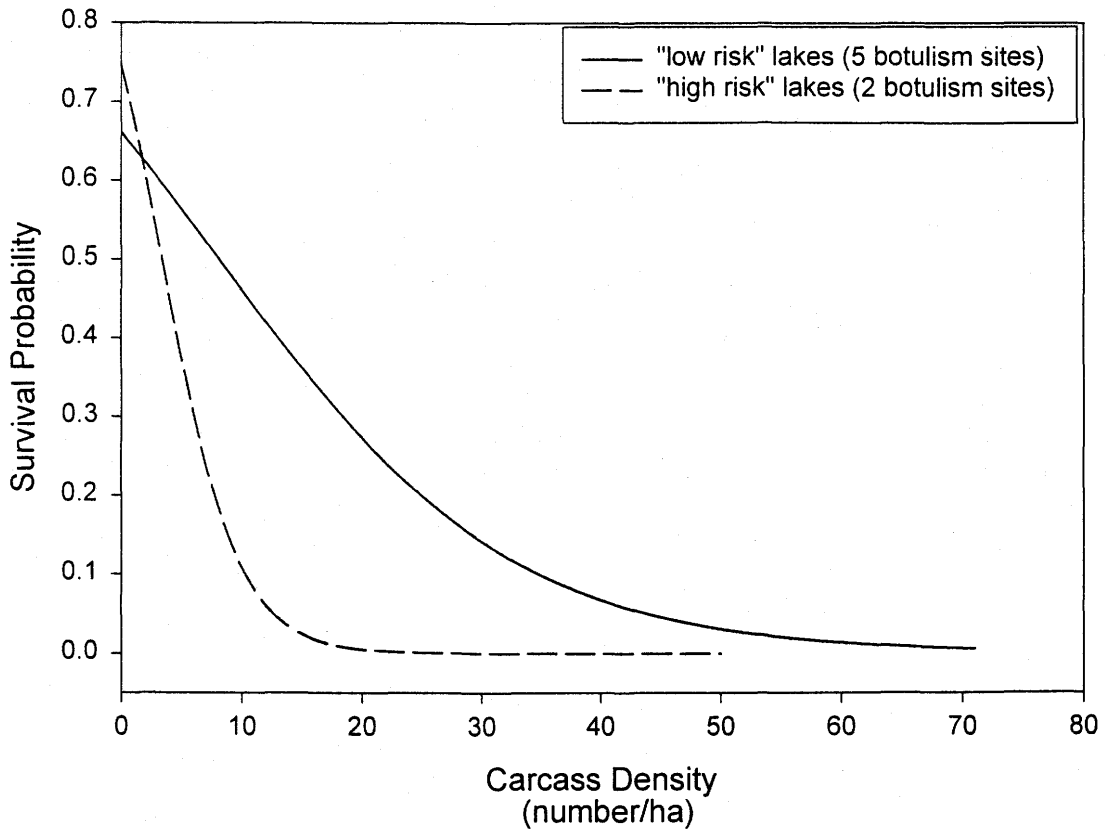

Figure 3.1. Survival probability of radio-marked mallards at 7 botulism outbreak sites in relation to carcass density (number of carcasses per hectare), 1999-2000. Survival probability is defined here as survival over a 24 hour period, that is, 1- P[alive at day *d* and dead at day *d*+1].
Coefficient(-2 Loglikelihood) = 56.411 H, 168.998 L.
Survival= 1.0832(SE=0.4440) -0.32(SE=0.089)density, 0-51, n=67 "high risk" lakes
Survival = 0.6665(SE=0.2830) -0.08(SE=-0.029)density, 0-71, n=130 "low risk" lakes

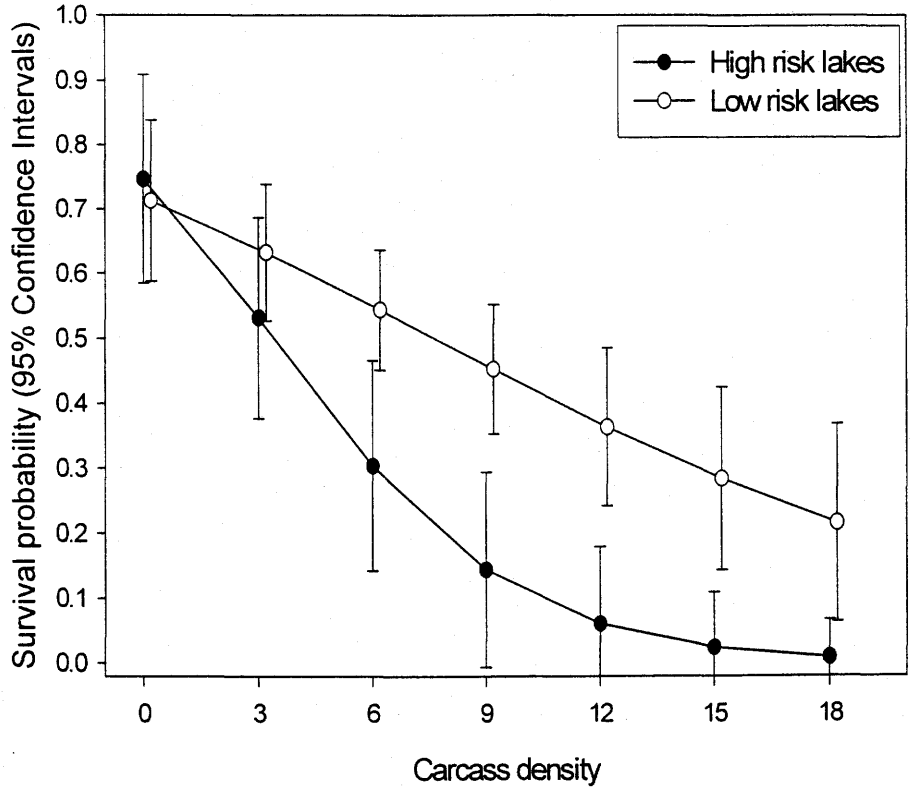

Figure 3.2. Survival probability of radio-marked mallards in relation to carcass density (carcasses/ha), on high and low risk lakes. Survival probability is defined here as survival over a 24 hour period, that is, 1- P[alive at day *d* and dead at day *d*+1].
Lines were plotted from equations:
Survival= 1.08(SE=0.444) -0.32(SE=0.089) density, 0-20, n=60, "high risk" lakes
Survival= 0.91(SE=0.317) -0.12(SE=0.037) density, 0-20, n=122, "low risk" lakes

lakes, carcasses with maggots were a better predictor of survival than carcasses without maggots (Table 3.3). The top two models considering only effects of carcass density ('maggots' and 'nomag') account for 90.4% of the model weight. The 'lake' variable had no support among low risk lakes consistent with previous modeling results (Table 3.2). The top model considering only maggoty carcasses had 67.3% model weight and was 2.9 times better supported by data than the second model (Table 3.3). I plotted the relationship of survival probability and carcass density separated by SOD description (Fig 3.3). Survival probability decreased the most as a result of maggoty carcasses versus all, and non-maggoty carcasses at the low risk lakes (Fig. 3.3). Thus, density SOD 4 to 6 carcasses was a better predictor of survival probability than densities of carcasses in earlier stages of decomposition, (SOD 1 to 3).

I also was interested in estimating total numbers of carcasses (and maggot-laden carcasses) found on wetlands with and without removal, so I pooled live and dead birds for each lake. Although density of maggoty carcasses appeared to be somewhat lower on removal lakes, there was wide variation in residual carcass density on lakes with removal (Table 3.4). Differences in total and maggoty carcass densities were compared among the five low risk lakes using analysis of covariance (PROC GLM, SAS Institute 1999), controlling for effects of search date and an interaction between date and removal treatment. Lakes where carcasses were removed tended ($F = 3.15$, $df = 1, 102$, $P = 0.08$) to have lower density of maggot-laden carcasses than did lakes without removal (least squares means [LS means]: 2.4/ha ± 0.9 [SE] versus 6.2/ha ± 1.0). Differences in total carcass density ($F = 2.89$, $df = 1, 102$, $P = 0.09$) were less pronounced (LS means: 6.0/ha ± 1.1 versus 7.8/ha ± 1.2, for removal and non-removal lakes, respectively).

3.4 DISCUSSION

3.4.1 Carcass density models

Contrary to expectations based on earlier published work, mortality was >50% even when average carcass densities were <4/ha. Survival was especially low at relatively

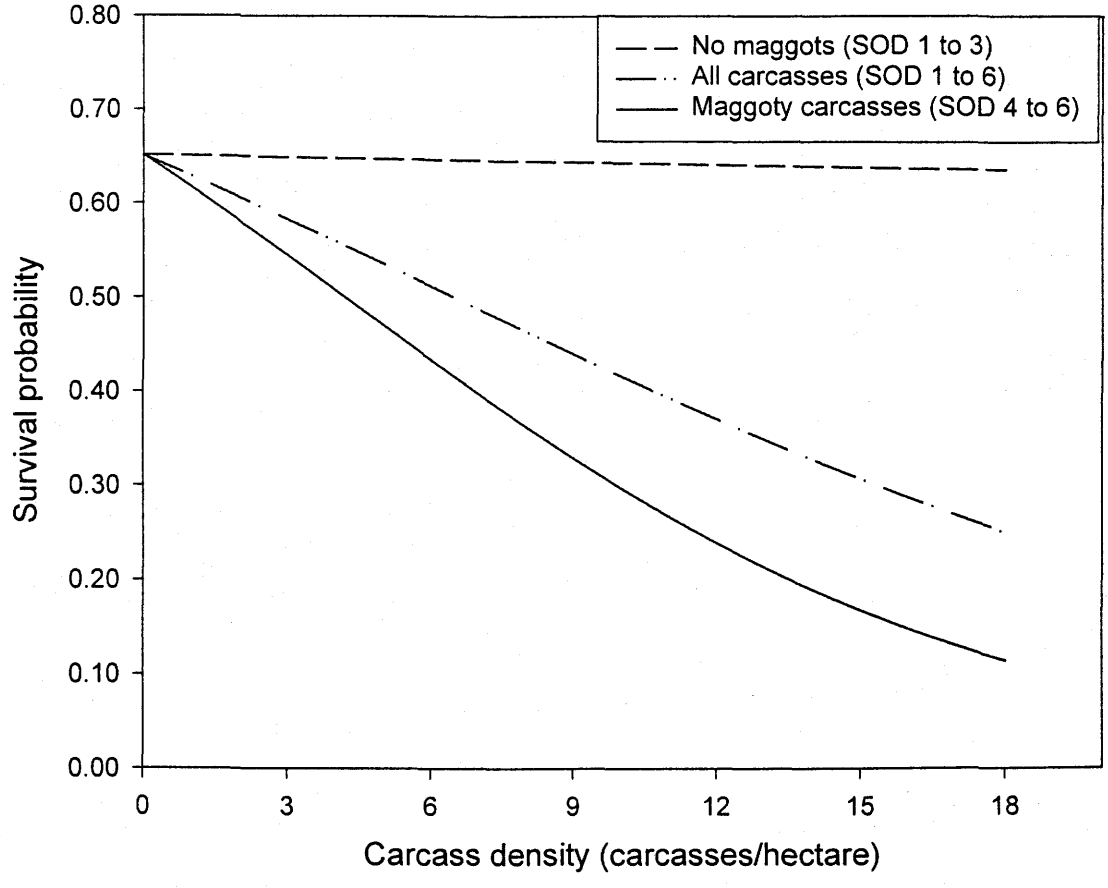

Figure 3.3. Survival probability of mallards in relation to carcass density on five low risk botulism lakes, 1999-2000. Carcasses were divided by Stage of Decomposition (SOD); SOD 1 to 3 lacked maggots whereas SOD 4 to 6 had maggots. Lines were plotted from these equations:
Survival= 0.62(SE=0.284) –0.0035(SE=0.0840) carcass density, n=115, SOD 1 to 3
Survival= 0.62(SE=0.284) –0.0957(SE=0.0380) carcass density, n=115, SOD 1 to 6
Survival= 0.62(SE=0.284) –0.1484(SE=0.0573) carcass density, n=115, SOD 4 to 6

Table 3.4. Total and maggot-laden carcass densities (carcasses/ha) near dead and live radio-marked mallards, 1999-2000. Shown are number of searches (N), mean carcass density ($\bar{x}$), and carcass density range (Range).

	All carcasses[a]			Maggoty carcasses[b]		
Lake	N	$\bar{x}$	Range	N	$\bar{x}$	Range
Whitewater[d]	42	10	0-39	34	4	0-28
Old Wives	26	11	0-51	NA[c]	-	-
Eyebrow	24	11	0-29	16	8	0-28
Crane	32	9	0-71	32	6	0-64
Frank[d]	42	7	0-21	42	3	0-9
Paysen[d]	16	7	0-38	16	4	0-20
Kettlehut	16	11	0-42	16	8	0-35

[a] Includes all carcasses found in search plots, (stages of decomposition [SOD] 1-6).

[b] Includes only carcasses in stages of decomposition with maggots present (SOD 4-6).

[c] Carcasses found in search plots were not recorded by SOD; therefore, data are not available.

[d] Carcass removal was conducted.

low carcass densities, particularly on high risk lakes. This means that when areas of residual maggot-laden carcass densities occur within a wetland, it reduces survival probability. Survival probability may vary with toxin toxicity, the amount of available toxin, or other as yet unidentified factors.

3.4.2 Survival probability

Survival probability decreased with increased carcass density for both groups of pooled lakes, with the rate of decrease being much steeper on high risk wetlands between carcass densities of 0 to 12 carcasses/ha (n=67 high risk lakes, n=130 low risk lakes) (Fig. 3.1). The relationship was evaluated with carcass density truncated to <20 carcasses/ha (n=60 high risk lakes, n=122 low risk lakes), and plotted with estimated survival and the 95% confidence intervals (Fig. 3.2). There was no overlap in confidence intervals among high and low risk lakes when carcass density was between 9 and 15. At 12 carcasses/ha, a bird was 6 times more likely to die at a high risk lake versus a low risk lake. Reed and Rocke (1992) reported that captive mallards in pens with 12 carcasses/ha were 4.5 times more likely to die of botulism than were birds in pens with no carcasses. By contrast, I found that wild ducks on high risk lakes were 12.2 times more likely to die at 12 carcasses/ha than at 0 carcasses/ha, and on low risk lakes were 2.0 times more likely to die.

I illustrated the apparent magnitude of the influence of carcass density on survival probability on both high and low risk lakes. Using binary regression parameter estimates, I estimated expected survival probability for an individual with an average carcass density on a high risk lake to be 0.144, whereas that for an individual with 50% fewer carcasses than average was 0.413. Thus, a 50% decrease in carcass density below the mean resulted in an estimated 27% increase in survival probability. The corresponding figure for an individual on a low risk lake was 13% (i.e., estimated survival probability increased from 0.462 to 0.594). Survival probability increased for birds on high and low risk lakes when carcass density was lowered, but the magnitude of change was different and likely depended on other factors that affect risk of death.

3.4.3 Maggoty carcasses

Density of SOD 4 to 6 carcasses had the greatest effect on survival probability (Fig. 3.3). Although SOD 6 carcasses had decayed to the point of having little remaining flesh and maggots, depending on decomposition rates, these carcasses may have been maggot rafts 1 or 2 days prior to when we found them in our searches. Therefore, SOD 6 carcasses could have represented a threat to radio-marked mallards 1-2 days earlier. At 18 carcasses/ha, expected survival probability is 64% for carcasses without maggots (SOD 1-3), 25% for all carcasses (SOD 1-6), and only 11% if density includes only maggoty carcasses on low risk lakes (Fig. 3.3). Density of maggoty carcasses was the best predictor of survival probability.

Although density of maggot-laden carcasses was lower on low risk lakes where dead birds were removed, maggot-laden carcasses were abundant in specific areas. Therefore, it is likely that survival of radio-marked birds was not enhanced by carcass removal (Chapter 2) because too many maggot-laden carcasses remained in some areas of lakes during removal operations (Table 3.4).

3.4.4 Carcass density thresholds

Carcass density thresholds existed, but vary with other yet unmeasured factors that affect "risk". The steepest incremental change in survival would occur if carcass densities were maintained <6-9 carcasses/ha across all locations on a high risk lake (Fig. 3.2). Removal operations would have limited impact on survival of healthy birds if areas of a high risk lake had >9 carcasses/ha. On low risk lakes, removal operations may achieve a ~10% incremental gain in survival with every 3 carcasses/ha reduction in carcass density. However, the cost and logistical feasibility of accomplishing those reductions seem overwhelming, especially on larger wetlands (Chapter 2).

3.4.5 Assumptions and limitations

I speculate that even intense search efforts did not find 100% of the carcasses, another reason why survival estimates were <<1.0 when no carcasses were found. These searches were generally more intense than those of removal operations, because crews

spent more time wading within a small area or the airboat thoroughly covered the predefined area. Some dead, radio-marked mallards were found at locations within wetlands that were extremely hard or impossible to access via airboat due to extremely dense vegetation in only a few centimeters of water. I also recognize that locations of search plots for dead birds may have not contained the exact area where the bird contracted botulism. Also, the searched areas of random live birds may not have entirely contained the area where the bird was actually spending most time. However, search locations of live radio-marked birds were usually in close proximity to these areas, and searches did reveal strong differences in carcass densities at dead versus live bird locations on "high" and "low" risk lakes.

3.4.6 Management recommendations

Although models clearly demonstrate that survival could potentially be increased with carcass removal, the logistics of achieving the desired carcass densities need to be considered. Wetland size and amount of emergent vegetation are important factors that influence the feasibility of maintaining low carcass densities during removal operations (Chapter 2). Removal operations on high risk wetlands will not effectively reduce mortality unless extremely low carcass densities can be maintained during an outbreak. Furthermore, the cost of intense removal operations needed to maintain low carcass densities for the amount of reduced mortality must be assessed. The number of birds exposed to a botulism outbreak (the population with which a removal operation could potentially reduce mortality) on Paysen lake is considerably less than that on Old Wives Lake.

The underlying cause of what made the lakes high or low risk lakes was not known. It could be related to different factors or a combination of factors related to the severity of the botulism outbreaks. Wobeser and Bollinger (2002) outline the basic factors involved in botulism outbreaks that include the presence of the bacterium and the bacteriophage, substrate or nutrient material to support bacterial growth and toxin production. Also, environmental conditions such as warm temperature and lack of oxygen suitable for bacterial growth and toxin production, "packaging" of toxin in a

form that will be consumed by birds, and the presence of susceptible birds that will consume the toxin. Given the lakes in 1999-2000, had these basic factors, and subsequently had botulism outbreaks, I can only speculate there were specific differences among wetlands that caused more severe outbreaks at the high risk lakes. The high risk lakes had more severe outbreaks than our low risk lakes and apparently than at the outbreaks documented by Reed and Rocke (1992) at the Sacramento National Wildlife Refuge, California. My study botulism wetlands had lower survival probability for waterfowl at lower carcass densities and outbreaks could persist within these wetlands even at very low maggot-laden carcass densities. Therefore, carcass removal operations are not recommended as a viable management technique.

CHAPTER 4. SYNTHESIS

Carcass removal operations have been the traditional and most advocated response to outbreaks of avian botulism in North America. This method recognizes the need to break the carcass-maggot cycle via carcass removal and thereby attempt to reduce mortality of waterfowl. It also considers the view that a potentially beneficial action is better than doing nothing, and visible action demonstrates good intentions (Peterson 1991). However, until this study, no one had tried to evaluate the effectiveness of carcass removal in increasing duck survival in an operational setting. My results provided no support for the hypothesis that carcass removal reduces duck mortality. Survival of molting mallards was no better on lakes with removal than on lakes with no management. Perhaps paradoxically, however, I also found that mallard survival probability was higher in areas of low carcass density, consistent with the notion that removing carcasses could have positive impacts on survival. A likely explanation for this apparent discrepancy hinges on the reasonable possibility that carcass density cannot be reduced sufficiently to very low levels in all areas of managed wetlands during outbreaks. Thus, despite best efforts, some areas of wetlands invariably harbor high carcass densities.

Other sources of variation in survival were related to date of radio-marking, sex, and mass. Survival probability decreased for birds radio-marked and released on dates closer to when peak mortality was estimated to have occurred on lakes in 1999. At Whitewater and Old Wives lakes, a sharp peak in mortality occurred during the second week of August, which coincided with the generally later arrival of female mallards. I also noticed more female pintails and females of other species dead during searches in mid August. There was also some evidence of lower female survival than males. Body mass was influential in survival probability in 2000. These could be inter-related, more closely tied to sex in 1999, because difference in mean sex-specific mass was greater owing to more breeding effort by females. Model results revealed differences

in survival among wetlands associated to the outbreak and possible characteristics related to the severity of the outbreak.

The cost of removal operations was provided and broken down as cost per duck and cost per hectare. Removal operations in 2000 and 2001 were much more intense on the smaller lakes. These costs do not include capital cost of airboats; while some boats were rented for intense removal purposes, most were owned by wildlife agencies. Of the five documented removal operations, the average cost for each duck removed was $877.86 and the average cost of removal per wetland hectare was $72.40. In general, cost/duck produced by Prairie Habitat Joint Venture (PHJV) programs is < $25.00.

I provided models of desired low carcass density thresholds that would need to be achieved and maintained across lakes to effectively increase survival (Chapter 3). High and low risk lakes were also identified. Whitewater and Old Wives lakes were identified as 'high' risk lakes. Given the size and vegetation characteristics of botulism-prone wetlands and cost of removals, the actual field removal attempts were not able to obtain and maintain low carcass densities. Removal operations conducted during the study resulted in no benefit to increased survival of radio-marked birds when compared among paired wetlands. Results of further modeling carcass density divided by stage of decomposition verified that maggoty carcasses are the best predictor of survival probability.

Future avian botulism research could be organized and planned as a group decision similar to this study. Decisions were made based on thorough communication following a management decision tree. Considerable annual mortality in waterfowl from botulism has occurred for decades and cannot be prevented. Although it is not yet known for sure, botulism mortality may have an adverse effect on waterfowl populations. So, until that information becomes known, the decision was made that effort to manage outbreaks was better than doing nothing. Therefore, Adaptive Resource Management (ARM) is being utilized by agencies to implement, evaluate, and re-plan management using the best information available. Resources and equipment were pooled together to launch the most effort possible towards testing the effectiveness of removal operations. Among the many questions to answer regarding botulism, the decision to evaluate removal

operations was a collective group decision made by many people. Based on the results of the three years (1999-2001) of cooperative investigations, future research decisions can be made as a team effort to re-plan and implement new management.

Future research needs to determine impacts of botulism mortality on waterfowl populations. Continued banding of botulism versus non-botulism lakes may help answer management questions and provide insight for the population dynamics of hosts impacted by the disease. Also, estimates of the number of birds exposed to botulism outbreaks are needed, and over time, these data in conjunction with other data such as our mortality estimates of mallards will help determine estimates of overall losses. Comparative studies could also be utilized among lakes and waterfowl species. These estimates could then be used to determine the role of botulism mortality on waterfowl populations. Learning whether botulism mortality has additive negative impacts or plays a more compensatory role on waterfowl populations would be useful to prioritize management decisions.

Work could specifically identify the frequency and magnitude of losses on the high-risk lakes and further identify differences among the high and low risk lakes. My models of survival (Chapter 2) and carcass density and its relationship on bird survival identified the high and low risk lakes (Chapter 3). However, we do not know why there was considerable survival variation among wetlands and years. Toxin production and transmission may be important factors related to the severity of botulism outbreaks. On high risk wetlands, survival remained low even at low carcass densities. This could mean the toxin is more toxic, or there is more toxin at these locations because lower doses are more efficient at killing birds than on low risk lakes. Therefore, an outbreak may be more likely to perpetuate and persist as a result of differences in characteristics of toxins or other factors.

Future research could also focus on other methods of managing outbreaks and could be carried out on smaller low risk wetlands. Techniques such as water-level or vegetation manipulation may be applicable on these wetlands. Eyebrow Lake has consistent annual waterfowl losses and is suitable for this kind of experimentation

because it is divided into several managed cells. Management could be implemented, evaluated, and re-planned following the pattern of this study.

Mortality from botulism tends to be grossly under estimated and often goes unnoticed. It is the primary disease that effects mallards during the post-breeding phase of the annual cycle (Samuel 1992), mainly during July through September. Reporting botulism outbreaks is paramount to understanding the distribution and frequency of epidemics. Further, accurate estimates of species composition and number of dead birds at an outbreak are needed; one or two point estimates can very useful in making future decisions regarding management of a lake. Blood samples from sick birds could be obtained to document botulism and learn more about the toxin from high and low risk lakes. Standard protocols and data sheets should be established among provinces for this to be achieved. Time and budgets could then be adjusted to fit this survey into annual routine monitoring. The significance of this disease in a specific host species could be an important factor limiting population growth, or in cases of severe outbreaks, result in catastrophic losses, especially in a species already struggling such as the northern pintail. Since these lakes are much larger and outbreaks often more severe, decisions for management of outbreaks may be different than on smaller wetlands.

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